REMARKS

Claims 26-29 are currently pending.

The Applicants respectfully thank the Examiner for a teleconference with the undersigned representative, Thomas S. Kim, on July 14, 2006, to discuss the present Final Office Action. In the teleconference, the Examiner clarified the outstanding rejections. As a result of the clarifications, Claims 26 and 27 are hereby amended by removing the phrase, "based on Clustal method of alignment." The objections directed to Claim 26 is now moot in light of the amendments. No new matter is added.

The pending claims stand rejected under 35 USC 112, first paragraph, for lack of enablement of the full scope of the claimed invention. Reconsideration and allowance are respectfully requested.

The rejection of claims 26-29 under 35 USC 112, first paragraph, for lack of enablement should be withdrawn. The combination of the disclosure in the specification and the knowledge in the art at the time of filing the instant application would enable one of ordinary skill to make and use the claimed invention directed to adenylate translocators without undue experimentation. The combined knowledge includes, *inter alia,* a test for translocator activity, high homology, and significant primary and secondary structure, which provides one of ordinary skill guidance as to which amino acid residues can be altered while maintaining protein function.

There is significant primary sequence information available for guidance, including homology and highly conserved motifs, which are consistent for adenylate translocators. Appendix A, attached herewith, is a Clustal alignment of the following three protein sequences using the default parameters cited in the specification:

1. SEQ ID NO:18 of the instant claims (wheat brittle-1 protein)
2. SEQ ID NO:21 of the instant specification (maize brittle-1 protein) and also found in Sullivan et al., *Plant Cell* 3:1337-1348 (1991) ("Sullivan"); NCBI GI No. 231654
3. mitochondrial energy transfer protein from potato; NCBI GI No. 4138581 (direct submission on June 8, 1996) (also referred to as a brittle-1 homolog on page 24-25 of the instant specification)

The alignment shows the close homology that exists between SEQ ID NO:18 and the known maize (SEQ ID NO:21; NCBI GI NO. 231654) and known potato sequences (NCBI GI No. 4138581). Appendix B, attached herewith, is provided to display the percent identity (and percent divergence in the lower half triangle) between the various sequences in Appendix A using the Clustal alignment method with the default parameters. SEQ ID NO:18 is shown to share 57.3% identity to the maize protein and 53.5 % identity to the potato protein.

In addition to this close homology, the alignment of Appendix A also shows significant conservation of motifs, which indicate that the protein is likely an adenylate transporter, and more particularly brittle-1. Sullivan (p. 1342, col. 2) and Palmieri F., *FEBS Lett.* 346:48-54 (1994) ("Palmieri") (p. 50, col. 2) report that maize brittle-1 belongs to the mitochondrial carrier family. This family of proteins is characterized as having a tripartite structure made up of repeating ~100 amino acid sequences, referred to herein as "100 aa repeat." Each 100 aa repeat sequence contains 2 hydrophobic stretches (Palmieri on p. 50, col. 1) and displays conserved elements within the 100 aa repeat. More specifically, Palmieri provides that a distinct feature of the 100 aa repeat is the sequence motif (referred to as "signature motif"): P-h-D/E-h-h-K/R-h-R/K-(20-30 amino acids)-D/E-G-(4 amino acids)-a-K/R-G (h = hydrophobic, a = aromatic). All the aligned sequences display three 100 aa repeat comprising the signature motif. The signature motifs are boxed in Appendix A (the 20-30 amino acid region is not boxed).[1]

Applicants further provide in Appendix C, attached herewith, a Clustal alignment of the three 100 aa repeats of the wheat brittle-1 protein (SEQ ID NO:18) (the three repeats are underlined in Appendix A). The three repeats were selected based on the signature motif (see, for example, Saraste et al., *FEBS Lett.* 144(2):250-254 (1982), specifically Table 1 found on page 251). The sequence motifs are boxed in Appendix C. Identical residues are shaded. Amino acids conserved in all three repeats are shown in the Consensus #1 sequence.

Applicants further provide in Appendix D, attached herewith, a table displaying the percent identity (and percent divergence in the lower half triangle) between the three corresponding 100 aa repeats of the wheat (SEQ ID NO:18),

[1] While there is high conservation in the signature motifs, Applicants note that there are minor substitutions at position 174, where histidine exists instead of arginine/lysine, and at position 366, where glutamine and histidine (potato sequence) exists instead of arginine/lysine, although both

maize (SEQ ID NO:21) and the potato sequence (individual alignments not shown). In summary, the percent identity of the 100 aa repeats between SEQ ID NO:18 and the maize and potato sequence are detailed in the following table:

Repeat	wheat (SEQ ID NO:18) versus maize (SEQ ID NO:21)	wheat (SEQ ID NO:18) versus potato
First 100 aa repeat	85.6	68.5
Second 100 aa repeat	83.7	65.2
Third 100 aa repeat	91.5	76.1

The high degree of sequence identity in these 100 aa repeats is a characteristic of adenylate transporters.[2]

Beyond the conserved primary structure, there is also significant conservation in secondary structure between the adenylate translocator proteins. The three repeating 100 aa repeats, discussed above, suggest that there are likely six transmembrane helices, two within each repeat. See, for example, Palmieri, on page 51, which discusses the validation of the conserved secondary structure in mitochondrial carrier family proteins and its significance.

In addition to the aforementioned structural characteristics described for brittle-1 proteins, provided are activity assays that one of ordinary skill can use to determine adenylate translocator activity. On page 6 of the present Final Office Action, the Patent Office incorrectly asserts that assaying the nucleic acid requires plant transformation. In fact, it has been shown that adenylate translocation activity can be determined *in vitro* by using isolated amyloplasts, as described in Shannon et al., *Plant Physiol.* 117:1235-1252 (1998) ("Shannon"). In particular, on page 1239, first column, Shannon details a method for determining translocator activity using intact amyloplast preparations. "All uptake studies were completed with intact amyloplast preparations and with lysed amyloplast preparations." On page 1245, Shannon discusses that brittle-1 mutants resulted in a 74% reduction in translocation activity as compared to wild type brittle-1. The results were

---

deviations are conservative substitutions. Additionally, the potato sequence substitutes a glycine for aspartic/glutamic acid at positions 169 and 291.

[2] Applicants submit that the higher level of conservation between wheat (SEQ ID NO:18) and maize (SEQ ID NO:21) is due to both being monocots; whereas potato is a dicot.

summarized on page 1246, first column, "the reduced [14C]Glc incorporation from ADP-Glc into starch by *bt1* amyloplasts was caused by the reduced transfer of ADP-Glc into the amyloplast." A skilled artisan equipped with this translocator assay could readily examine any alterations or mutations to the disclosed polypeptides of the present invention and confirm that function is maintained, even without the transformation of plants.

One of ordinary skill in the art, equipped with the provided structural information characteristic of mitochondrial carrier proteins (or more specifically, adenylate translocators) and the test to determine translocator activity, is able to practice the claimed invention without undue experimentation.

Please charge any fees or credit any overpayment of fees which are required in connection with the filing of this Response After Final to Deposit Account No. 04-1928 (E. I. du Pont de Nemours and Company).

Allowance of the above-referenced application is respectfully requested in view of the foregoing.

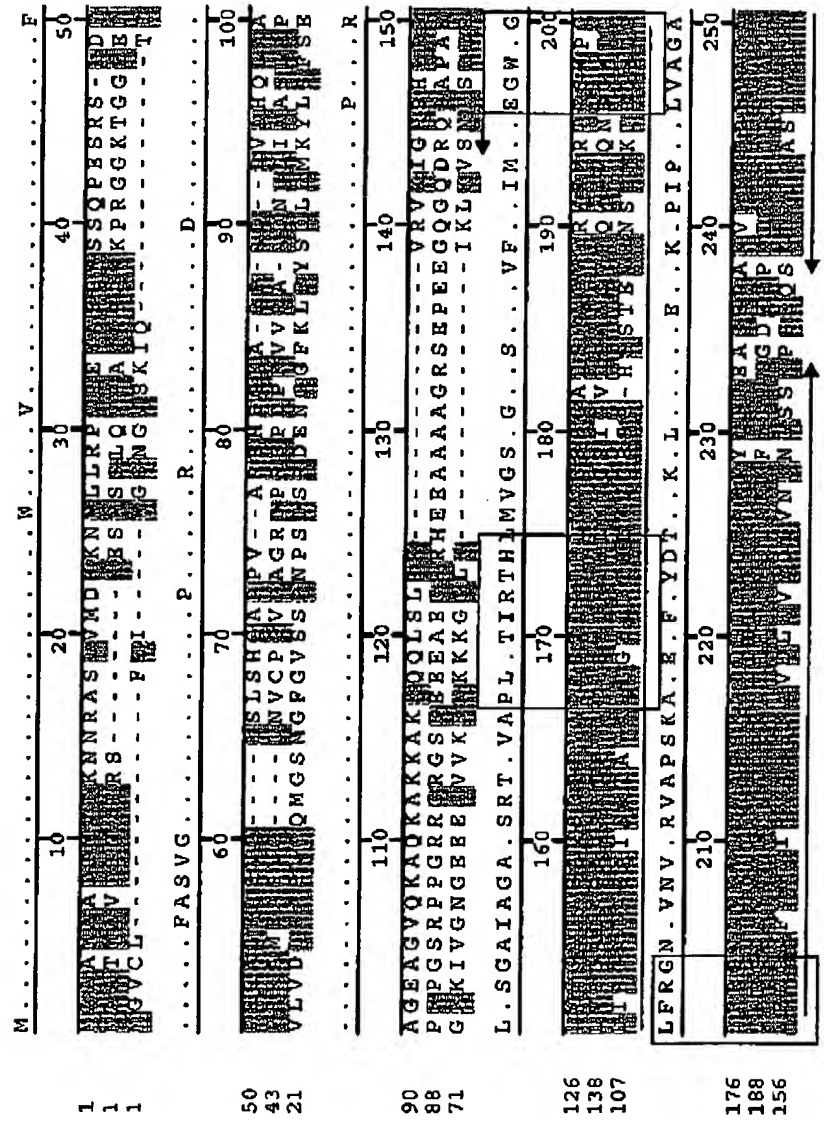
Respectfully submitted,

THOMAS S. KIM
AGENT FOR APPLICANTS
REGISTRATION NO. 51,009
TELEPHONE: 302-992-4061
FACSIMILE: 302-892-1026

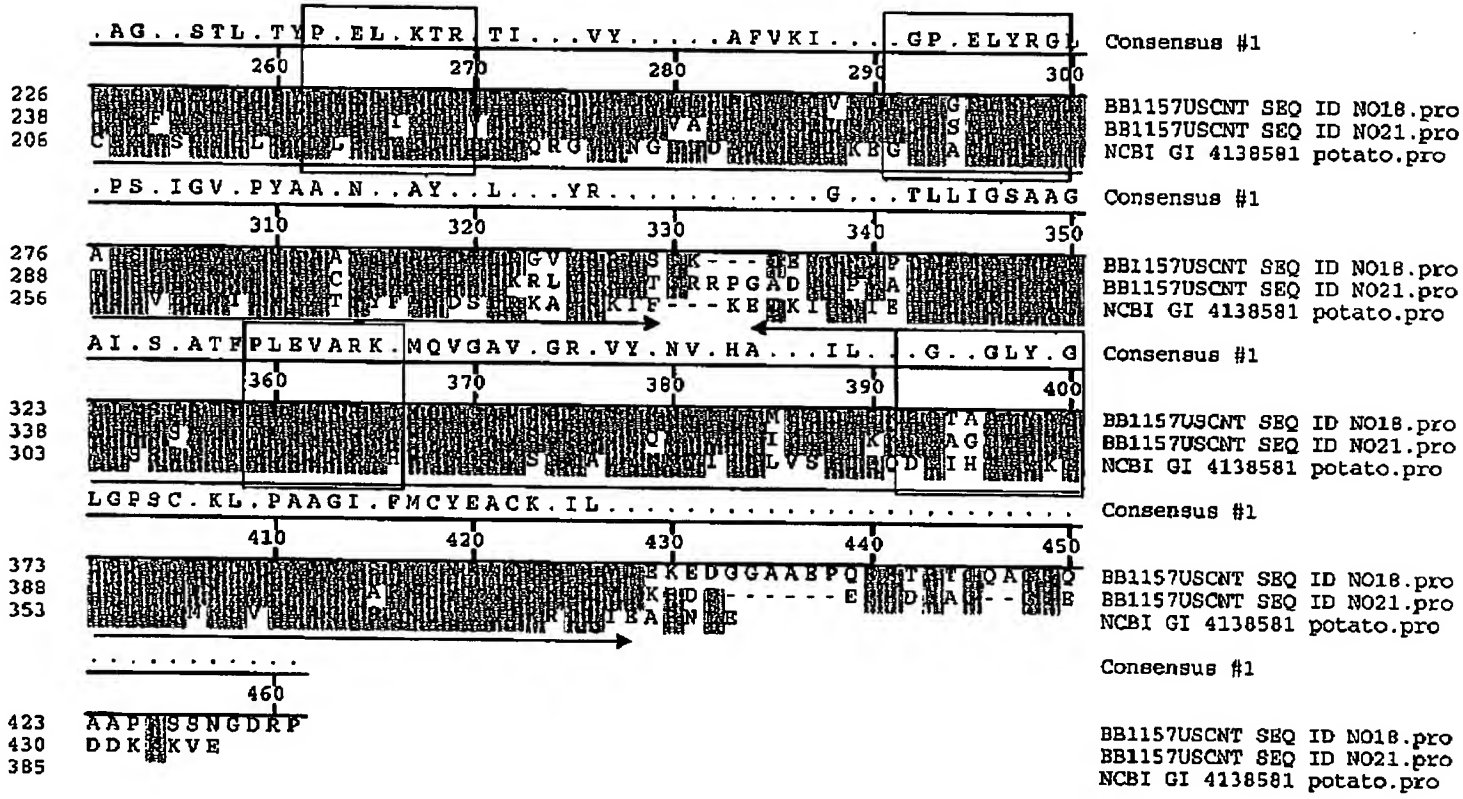
Dated: 8/31/06

Application No.: 10/659,199
Docket No.: BB1157USCNT

Appendix A (page 1 of 2)



Appendix A (page 2 of 2)



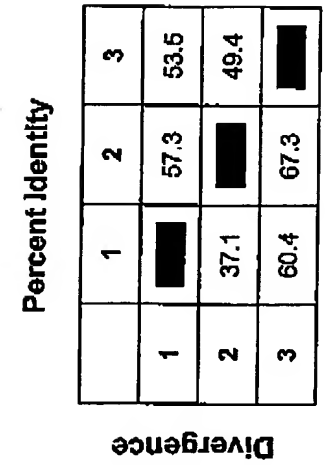
Shade (with black at 40% fill) residues that match the Consensus Sequence exactly.

The sequence motifs are boxed (P-h-D/E-h-h-K/R-h-R/K-(20-30 amino acids)-D/E-G-(4 amino acids)-a-K/R-G (h = hydrophobic, a = aromatic) (the 20-30 amino acid region is not boxed).

For SEQ ID NO:18, the three 100 aa repeats associated with mitochondrial carrier proteins are underlined with arrows at the start/stop.

Application No.: 10/659,199
Docket No.: BB1157USCNT

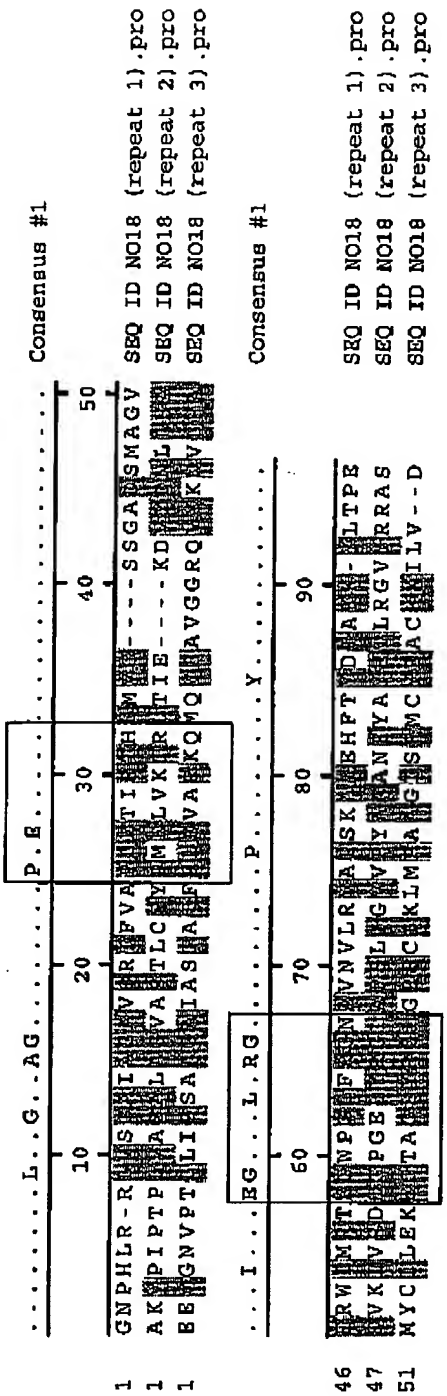
Appendix B



Application No.: 10/659,199

Docket No.: BB1157USCNT

Appendix C



Application No.: 10/659,199
Docket No.: BB1157USCNT

Appendix D

Percent Identity

Divergence	1	2	3
1		85.6	68.5
2	16.1		65.2
3	35.2	40.7	

SEQ ID NO18 (wheat brittle-1) repeat #1
SEQ ID NO21 (maize) (NCBI GI No. 231654) repeat #1
potato (NCBI GI No. 4138581) repeat #1

Percent Identity

Divergence	1	2	3
1		83.7	65.2
2	18.4		62.0
3	46.5	52.7	

SEQ ID NO18 (wheat brittle-1) repeat #2
SEQ ID NO21 (maize) (NCBI GI No. 231654) repeat #2
potato (NCBI GI No. 4138581) repeat #2

Percent Identity

Divergence	1	2	3
1		91.5	76.1
2	9.0		78.1
3	28.9	35.3	

SEQ ID NO18 (wheat brittle-1) repeat #3
SEQ ID NO21 (maize) (NCBI GI No. 231654) repeat #3
potato (NCBI GI No. 4138581) repeat #3